10/586245 1 IAP11 Rec'd PCT/PTO 1/1 JUL 2006

SEQUENCE LISTING

<110> Consejo Superior de Investigaciones Científicas

<120> GENERATION OF SPECIFIC ADHESION IN GRAM-NEGATIVE BACTERIA BY MEANS OF FIXING IMMUNOGLOBULIN SINGLE DOMAINS ON THEIR SURFACE WITH AUTOTRANSPORTERS

```
P1375PC
<130>
<150> ES P200400073
      2004-01-14 (January 14, 2004)
<151>
       10
<160>
       PatentIn version 3.1
<170>
<210>
       1
<211> 5587
       DNA
<212>
<213> Artificial
       DNA sequence of plasmid pVamyß
<223>
<400> 1
accegacace ategaatgge geaaaacett tegeggtatg geatgatage geeeggaaga
                                                                       60
                                                                       120
gagtcaattc agggtggtga atgtgaaacc agtaacgtta tacgatgtcg cagagtatgc
 cggtgtctct tatcagaccg tttcccgcgt ggtgaaccag gccagccacg tttctgcgaa
                                                                       180
 aacgcgggaa aaagtggaag cggcgatggc ggagctgaat tacattccca accgcgtggc
                                                                       240
 acaacaactg gcgggcaaac agtcgttgct gattggcgtt gccacctcca gtctggccct
                                                                       300
 gcacgcgccg tcgcaaattg tcgcggcgat taaatctcgc gccgatcaac tgggtgccag
                                                                       360
 cgtggtggtg tcgatggtag aacgaagcgg cgtcgaagcc tgtaaagcgg cggtgcacaa
                                                                       420
 tcttctcgcg caacgcgtca gtgggctgat cattaactat ccgctggatg accaggatgc
                                                                       480
 cattgctgtg gaagctgcct gcactaatgt tccggcgtta tttcttgatg tctctgacca
                                                                       540
 gacacccatc aacagtatta ttttctccca tgaagacggt acgcgactgg gcgtggagca
                                                                       600
 tctggtcgca ttgggtcacc agcaaatcgc gctgttagcg ggcccattaa gttctgtctc
                                                                       660
                                                                       720
 ggcgcgtctg cgtctggctg gctggcataa atatctcact cgcaatcaaa ttcagccgat
                                                                       780
 agcggaacgg gaaggcgact ggagtgccat gtccggtttt caacaaacca tgcaaatgct
                                                                       840
 gaatgagggc atcgttccca ctgcgatgct ggttgccaac gatcagatgg cgctgggcgc
                                                                       900
 aatgcgcgcc attaccgagt ccgggctgcg cgttggtgcg gacatctcgg tagtgggata
                                                                       960
 cgacgatacc gaagacagct catgttatat cccgccgtta accaccatca aacaggattt
                                                                      1020
 tcgcctgctg gggcaaacca gcgtggaccg cttgctgcaa ctctctcagg gccaggcggt
                                                                      1080
 gaagggcaat cagctgttgc ccgtctcact ggtgaaaaga aaaaccaccc tggcgcccaa
                                                                      1140
 tacgcaaacc gcctctcccc gcgcgttggc cgattcatta atgcagctgg cacgacaggt
                                                                       1200
 ttcccgactg gaaagcgggc agtgagcggt acccgataaa agcggcttcc tgacaggagg
                                                                       1260
  ccgttttgtt ttgcagccca cctcaacgca attaatgtga gttagctcac tcattaggca
                                                                       1320
  ccccaggctt tacactttat gcttccggct cgtatgttgt gtggaattgt gagcggataa
                                                                       1380
  caatttcaca caggaaacag ctatgaccat gattacgaat ttctagataa cgagggcaaa
                                                                       1440
  tcatgaaata cctattgcct acggcagccg ctggattgtt attactcgcg gcccagccgg
                                                                       1500
  ccatggctca ggtgcagctg gtggagtctt ggggaggctc ggtgcaggct ggggggtctc
                                                                       1560
  tgagactctc ctgcacagcc cctggattca cctccaatag ctgccgcatg gactggtacc
                                                                       1620
  gccaggctgc agggaagcag cgcgagtggg tctcatctat tagtactgat ggtcgcacaa
                                                                       1680
  gctatgcaga ctccgtgaag ggccgattca ccatctccaa agacaaagcc aaggacacgg
  tgtatctgca aatgaacagc ctgaaacctg aggacacggc catctattac tgtgccgtga
                                                                       1740
                                                                       1800
  ggacgaatgg gtatcgtccg caatctcacg aatttcgcta ctggggcccg gggacccagg
                                                                       1860
  tcaccgtctc ctcagcggcc gcggcgtcgg gggccgaatt cgtcgacggt gcgccggtgc
                                                                       1920
  cgtatccgga tccgctggaa ccgatcgaca attcagccgc aattagtatg gcaaatccac
                                                                       1980
  gtccaccaac accgcgggtc gctgcggccg tattttcatt ggatgattat gatgcaaaag
                                                                       2040
  acaatagtga atcatcaata ggtaatttag ctcgtgtaat acctagaatg ggaagggagt
                                                                       2100
  taattaatga ttatgaagaa atccccttgg aggagttgga agatgaagcg gaagaagaac
```

2160 gtcgccaagc aacgcaattc cactccaaaa gtcgtaaccg tagagctata tcatcggaac 2220 catcatctga tgaagatgca tctgaatcgg tttccacatc agacaaacac cctcaagata 2280 atacggaact tcatgaaaaa gttgagacgg cgggtttaca accaagagcc gcgcagccgc 2340 gaacccaage egeegeaa geegatgeag teageaceaa tactaacteg getttatetg 2400 acgcaatggc aagcacgcaa tctatcttgt tggatacagg tgcttactta acacggcaca 2460 ttgcacaaaa atcacgcgct gatgccgaaa aaaacagtgt ttggatgtca aacaccggtt 2520 atggccgtga ttatgcttcc gcacaatatc gccggtttag ttcgaaacgc acgcaaacac 2580 aaatcggcat tgaccgcagc ttgtccgaaa atatgcagat aggcggagta ttgacttact 2640 ctgacagtca gcatactttt gatcaggcgg gcggcaaaaa tacttttgtg caagccaacc 2700 tttatggtaa gtattattta aatgatgctt ggtatgtggc cggcgatatt ggtgcgggca 2760 gcttgagaag ccggttacaa acgcagcaaa aagcaaactt taaccgaaca agcatccaaa 2820 ccggccttac tttgggcaat acgctgaaaa tcaatcaatt cgagattgtc cctagtgcgg 2880 gtatccgtta cagccgcctg tcatctgcag attacaagtt gggtgacgac agtgttaaag taagttctat ggcagtgaaa acactaacgg ccggactgga ttttgcttat cggtttaaag 2940 3000 tcggcaacct taccgtaaaa cccttgttat ctgcagctta ctttgccaat tatggcaaag 3060 gcggcgtgaa tgtgggcggt aaatccttcg cctataaagc agataatcaa cagcaatatt 3120 cagcaggcgt cgcgttactg taccgtaatg ttacattaaa cgtaaatggc agtattacaa 3180 aaggaaaaca attggaaaaa caaaaatccg gacaaattaa aatacagatt cgtttctaaa 3240 3300 aaatggcgca cattgtgcga cattttttt gtctgccgtt taccgctact gcgtcacgga 3360 tececaegeg ecetgtageg gegeattaag egeggegggt gtggtggtta egegeagegt gaccgctaca cttgccagcg ccctagcgcc cgctcctttc gctttcttcc cttccttct 3420 3480 cgccacgttc gccggctttc cccgtcaagc tctaaatcgg ggcatccctt tagggttccg 3540 atttagtgct ttacggcacc tcgaccccaa aaaacttgat tagggtgatg gttcacgtag 3600 tgggccatcg ccctgataga cggtttttcg ccctttgacg ttggagtcca cgttctttaa 3660 tagtggactc ttgttccaaa ctggaacaac actcaaccct atctcggtct attcttttga 3720 tttataaggg attttgccga tttcggccta ttggttaaaa aatgagctga tttaacaaaa 3780 atttaacgcg aattttaaca aaatattaac gtttacaatt tcaggtggca cttttcgggg aaatgtgcgc ggaaccccta tttgtttatt tttctaaata cattcaaata tgtatccgct 3840 catgtcgaga cgttgggtga ggttccaact ttcaccataa tgaaataaga tcactaccgg 3900 3960 gcgtattttt tgagttatcg agattttcag gagctaagga agctaaaatg gagaaaaaaa 4020 tcactggata taccaccgtt gatatatccc aatggcatcg taaagaacat tttgaggcat 4080 ttcagtcagt tgctcaatgt acctataacc agaccgttca gctggatatt acggcctttt 4140 taaagaccgt aaagaaaaat aagcacaagt tttatccggc ctttattcac attcttgccc gcctgatgaa tgctcatccg gagttccgta tggcaatgaa agacggtgag ctggtgatat 4200 4260 gggatagtgt tcacccttgt tacaccgttt tccatgagca aactgaaacg ttttcatcgc 4320 tctggagtga ataccacgac gatttccggc agtttctaca catatattcg caagatgtgg cgtgttacgg tgaaaacctg gcctatttcc ctaaagggtt tattgagaat atgtttttcg 4380 4440 tctcagccaa tccctgggtg agtttcacca gttttgattt aaacgtggcc aatatggaca 4500 acttcttcgc ccccgttttc accatgggca aatattatac gcaaggcgac aaggtgctga 4560 tgccgctggc gattcaggtt catcatgccg tctgtgatgg cttccatgtc ggcagaatgc 4620 ttaatgaatt acaacagtac tgcgatgagt ggcagggcgg ggcgtaattt ttttaaggca 4680 gttattggtg cccttaaacg cctggtgcta cgcctgaata agtgataata agcggatgaa 4740 4800 gccgcttatg tctattgctg gtttaccggt ttattgacta ccggaagcag tgtgaccgtg 4860 tgcttctcaa atgcctgagg ccagtttgct caggctctcc ccgtggaggt aataattgct 4920 cgacatgacc aaaatccctt aacgtgagtt ttcgttccac tgagcgtcag accccgtaga 4980 aaagatcaaa ggatcttctt gagatccttt ttttctgcgc gtaatctgct gcttgcaaac 5040 aaaaaaacca ccgctaccag cggtggtttg tttgccggat caagagctac caactctttt 5100 tccgaaggta actggcttca gcagagcgca gataccaaat actgtccttc tagtgtagcc 5160 gtagttaggc caccacttca agaactctgt agcaccgcct acatacctcg ctctgctaat 5220 cctgttacca gtggctgctg ccagtggcga taagtcgtgt cttaccgggt tggactcaag 5280 acgatagtta ccggataagg cgcagcggtc gggctgaacg gggggttcgt gcacacagcc 5340 cagcttggag cgaacgacct acaccgaact gagataccta cagcgtgagc tatgagaaag 5400 cgccacgctt cccgaaggga gaaaggcgga caggtatccg gtaagcggca gggtcggaac 5460 aggagagcgc acgagggagc ttccaggggg aaacgcctgg tatctttata gtcctgtcgg 5520 gtttcgccac ctctgacttg agcgtcgatt tttgtgatgc tcgtcagggg ggcggagcct 5580 atggaaaaac gccagcaacg cggccttttt acggttcctg gccttttgct ggccttttgc 5587 tcacatg

```
<210> 2
<211> 5563
<212> DNA
<213> Artificial
```

<223> DNA sequence of plasmid pVLMB10β

<400> 2

```
60
accegacace ategaatgge geaaaacett tegeggtatg geatgatage geeeggaaga
                                                                      120
gagtcaattc agggtggtga atgtgaaacc agtaacgtta tacgatgtcg cagagtatgc
                                                                      180
cggtgtctct tatcagaccg tttcccgcgt ggtgaaccag gccagccacg tttctgcgaa
                                                                      240
aacgcgggaa aaagtggaag cggcgatggc ggagctgaat tacattccca accgcgtggc
                                                                      300
acaacaactg gcgggcaaac agtcgttgct gattggcgtt gccacctcca gtctggccct
                                                                      360
gcacgcgccg tcgcaaattg tcgcggcgat taaatctcgc gccgatcaac tgggtgccag
                                                                      420
cgtggtggtg tcgatggtag aacgaagcgg cgtcgaagcc tgtaaagcgg cggtgcacaa
                                                                      480
tcttctcgcg caacgcgtca gtgggctgat cattaactat ccgctggatg accaggatgc
                                                                      540
cattgctgtg gaagctgcct gcactaatgt tccggcgtta tttcttgatg tctctgacca
                                                                      600
gacacccatc aacagtatta ttttctccca tgaagacggt acgcgactgg gcgtggagca
                                                                      660
tetggtegea ttgggteace ageaaatege getgttageg ggeeeattaa gttetgtete
                                                                      720
ggcgcgtctg cgtctggctg gctggcataa atatctcact cgcaatcaaa ttcagccgat
                                                                      780
agcggaacgg gaaggcgact ggagtgccat gtccggtttt caacaaacca tgcaaatgct
                                                                      840
gaatgagggc atcgttccca ctgcgatgct ggttgccaac gatcagatgg cgctgggcgc
                                                                      900
aatgcgcgcc attaccgagt ccgggctgcg cgttggtgcg gacatctcgg tagtgggata
                                                                      960
cgacgatacc gaagacagct catgttatat cccgccgtta accaccatca aacaggattt
                                                                     1020
togoctgotg gggcaaacca gogtggaccg ottgotgcaa otototoagg gocaggoggt
                                                                     1080
gaagggcaat cagctgttgc ccgtctcact ggtgaaaaga aaaaccaccc tggcgcccaa
tacgcaaacc gcctctcccc gcgcgttggc cgattcatta atgcagctgg cacgacaggt
                                                                     1140
                                                                     1200
ttcccgactg gaaagcgggc agtgagcggt acccgataaa agcggcttcc tgacaggagg
                                                                     1260
ccgttttgtt ttgcagccca cctcaacgca attaatgtga gttagctcac tcattaggca
                                                                     1320
ccccaggett tacactttat getteegget egtatgttgt gtggaattgt gageggataa
                                                                     1380
caatttcaca caggaaacag ctatgaccat gattacgaat ttctagagga gccttttttt
                                                                     1440
tggagatttt caacgtgaaa aaattattat tcgcaattcc tttagttgtt cctttctatt
                                                                     1500
ctcacagtgc acttgaaacg acactcacgc agtctccact ctccctgtcc gtcacccctg
                                                                     1560
gagagtcggc ctccatctcc tgcaggtata gtcagagcct cttccacagg aattggaaaa
                                                                     1620
cctgggtgga ttggtacctg cagaagccag ggcagtctcc acaagtcctg atctatgcgg
                                                                     1680
cttctattcg ggcctccggc gtccctgaca ggttcagtgg cagtgcttca ggcacagatt
                                                                     1740
ttacactgaa aatcagcagg gtggaggctg aggatgttgg ggtttattac tgcatgcaag
                                                                     1800
gtacacaccc gtacactttt ggccagggga ccaagctgac cgtcctaggt gcggccgcgg
                                                                     1860
cgtcgggggc cgaattcgtc gacggtgcgc cggtgccgta tccggatccg ctggaaccga
                                                                     1920
tcgacaattc agccgcaatt agtatggcaa atccacgtcc accaacaccg cgggtcgctg
                                                                     1980
cggccgtatt ttcattggat gattatgatg caaaagacaa tagtgaatca tcaataggta
                                                                     2040
atttagctcg tgtaatacct agaatgggaa gggagttaat taatgattat gaagaaatcc
                                                                     2100
ccttggagga gttggaagat gaagcggaag aagaacgtcg ccaagcaacg caattccact
                                                                     2160
ccaaaagtcg taaccgtaga gctatatcat cggaaccatc atctgatgaa gatgcatctg
                                                                     2220
aatcggtttc cacatcagac aaacaccctc aagataatac ggaacttcat gaaaaagttg
                                                                     2280
agacggcggg tttacaacca agagccgcgc agccgcgaac ccaagccgcc gcgcaagccg
atgcagtcag caccaatact aactcggctt tatctgacgc aatggcaagc acgcaatcta
                                                                     2340
                                                                     2400
tcttgttgga tacaggtgct tacttaacac ggcacattgc acaaaaatca cgcgctgatg
                                                                     2460
ccgaaaaaaa cagtgtttgg atgtcaaaca ccggttatgg ccgtgattat gcttccgcac
                                                                     2520
aatatcgccg gtttagttcg aaacgcacgc aaacacaaat cggcattgac cgcagcttgt
                                                                     2580
ccgaaaatat gcagataggc ggagtattga cttactctga cagtcagcat acttttgatc
                                                                     2640
aggcgggcgg caaaaatact tttgtgcaag ccaaccttta tggtaagtat tatttaaatg
                                                                     2700
atgcttggta tgtggccggc gatattggtg cgggcagctt gagaagccgg ttacaaacgc
                                                                     2760
agcaaaaagc aaactttaac cgaacaagca tccaaaccgg ccttactttg ggcaatacgc
                                                                     2820
tgaaaatcaa tcaattcgag attgtcccta gtgcgggtat ccgttacagc cgcctgtcat
                                                                     2880
ctgcagatta caagttgggt gacgacagtg ttaaagtaag ttctatggca gtgaaaacac
                                                                     2940
taacggccgg actggatttt gcttatcggt ttaaagtcgg caaccttacc gtaaaaccct
                                                                     3000
tgttatctgc agcttacttt gccaattatg gcaaaggcgg cgtgaatgtg ggcggtaaat
```

ccttcgccta taaagcagat aatcaacagc aatattcagc aggcgtcgcg ttactgtacc

3060

```
3120
gtaatgttac attaaacgta aatggcagta ttacaaaagg aaaacaattg gaaaaacaaa
                                                                     3180
aatccggaca aattaaaata cagattcgtt tctaaaatac caaattcata gcaaaataaa
                                                                     3240
atgccgtctg aactcaagct tgacctgtga agtgaaaaat ggcgcacatt gtgcgacatt
                                                                     3300
ttttttgtct gccgtttacc gctactgcgt cacggatccc cacgcgccct gtagcggcgc
                                                                     3360
attaagcgcg gcgggtgtgg tggttacgcg cagcgtgacc gctacacttg ccagcgccct
                                                                     3420
agcgcccgct cctttcgctt tcttcccttc ctttctcgcc acgttcgccg gctttccccg
                                                                     3480
tcaagctcta aatcggggca tccctttagg gttccgattt agtgctttac ggcacctcga
                                                                     3540
ccccaaaaaa cttgattagg gtgatggttc acgtagtggg ccatcgccct gatagacggt
                                                                     3600
ttttcgccct ttgacgttgg agtccacgtt ctttaatagt ggactcttgt tccaaactgg
                                                                     3660
aacaacactc aaccctatct cggtctattc ttttgattta taagggattt tgccgatttc
                                                                     3720
ggcctattgg ttaaaaaatg agctgattta acaaaaattt aacgcgaatt ttaacaaaat
                                                                     3780
attaacgttt acaatttcag gtggcacttt tcggggaaat gtgcgcggaa cccctatttg
                                                                     3840
tttatttttc taaatacatt caaatatgta tccgctcatg tcgagacgtt gggtgaggtt
                                                                     3900
ccaactttca ccataatgaa ataagatcac taccgggcgt attttttgag ttatcgagat
                                                                     3960
tttcaggagc taaggaagct aaaatggaga aaaaaatcac tggatatacc accgttgata
                                                                     4020
tatcccaatg gcatcgtaaa gaacattttg aggcatttca gtcagttgct caatgtacct
                                                                     4080
ataaccagac cgttcagctg gatattacgg cctttttaaa gaccgtaaag aaaaataagc
                                                                     4140
acaagtttta tccggccttt attcacattc ttgcccgcct gatgaatgct catccggagt
                                                                     4200
tccgtatggc aatgaaagac ggtgagctgg tgatatggga tagtgttcac ccttgttaca
                                                                     4260
ccgttttcca tgagcaaact gaaacgtttt catcgctctg gagtgaatac cacgacgatt
                                                                     4320
tccggcagtt tctacacata tattcgcaag atgtggcgtg ttacggtgaa aacctggcct
                                                                     4380
atttccctaa agggtttatt gagaatatgt ttttcgtctc agccaatccc tgggtgagtt
                                                                     4440
tcaccagttt tgatttaaac gtggccaata tggacaactt cttcgccccc gttttcacca
                                                                     4500
tgggcaaata ttatacgcaa ggcgacaagg tgctgatgcc gctggcgatt caggttcatc
                                                                     4560
atgccgtctg tgatggcttc catgtcggca gaatgcttaa tgaattacaa cagtactgcg
                                                                     4620
atgagtggca gggcggggcg taatttttt aaggcagtta ttggtgccct taaacgcctg
                                                                     4680
gtgctacgcc tgaataagtg ataataagcg gatgaatggc agaaattcga aagcaaattc
                                                                     4740
gacccggtcg tcggttcagg gcagggtcgt taaatagccg cttatgtcta ttgctggttt
                                                                     4800
accggtttat tgactaccgg aagcagtgtg accgtgtgct tctcaaatgc ctgaggccag
                                                                     4860
tttgctcagg ctctccccgt ggaggtaata attgctcgac atgaccaaaa tcccttaacg
                                                                     4920
tgagttttcg ttccactgag cgtcagaccc cgtagaaaag atcaaaggat cttcttgaga
                                                                     4980
teetttttt etgegegtaa tetgetgett geaaacaaaa aaaccacege taccageggt
                                                                     5040
ggtttgtttg ccggatcaag agctaccaac tctttttccg aaggtaactg gcttcagcag
                                                                     5100
agcgcagata ccaaatactg tccttctagt gtagccgtag ttaggccacc acttcaagaa
                                                                     5160
ctctgtagca ccgcctacat acctcgctct gctaatcctg ttaccagtgg ctgctgccag
                                                                     5220
tggcgataag tcgtgtctta ccgggttgga ctcaagacga tagttaccgg ataaggcgca
gcggtcgggc tgaacggggg gttcgtgcac acagcccagc ttggagcgaa cgacctacac
                                                                     5280
                                                                     5340
cgaactgaga tacctacagc gtgagctatg agaaagcgcc acgcttcccg aagggagaaa
                                                                     5400
ggcggacagg tatccggtaa gcggcagggt cggaacagga gagcgcacga gggagcttcc
agggggaaac gcctggtatc tttatagtcc tgtcgggttt cgccacctct gacttgagcg
                                                                     5460
                                                                     5520
tcgatttttg tgatgctcgt caggggggcg gagcctatgg aaaaacgcca gcaacgcggc
                                                                     5563
ctttttacgg ttcctggcct tttgctggcc ttttgctcac atg
<210> 3
<211> 47
<212> DNA
<213> Artificial
<223>
     Primer VHHA1
```

<210> 4 <211> 21 <212> DNA <213> Artificial

ctatgcggcc cagccggcca tggctcaggt gcagctggtg gagtctt

<400> 3

47

<223>	Primer GEN III-Rev	
<400>	4	
	atag ttagcgtaac g	21
<210>	5	
<211>	4 4	
<212>	DNA	
<213>	Artificial	
<223>	Primer Linker-A48-VamyA	
<400>		
	5	44
ggcggtccga ctgctaactc tggacaggtg cagctggtgg agtc 44		
<210>	6	
<211>	30	
<212>	DNA	
<213>	Artificial	
<223>	Primer Vamy-Not	
<400>	6	
	tct gcggccgctg aggagacggt	30
<i>J</i>		
<210>	7	
<211>	60	
<212>	DNA	
<213>	Artificial	
<223>	Primer Linker-A48	
<400>	7	
	' ctc acaactccca ccaggttcca tccgcaggcg gtccgactgc taactctgga	60
accedence academical coaggines and ecogological geological cademical grant and ecogological coaggines		
<210>	8	
<211>	37	
<212>	DNA	
<213>	Artificial	
<223>	Primer Linker -A48-Vamy-eag1	
<400>	Ω	
	o cgcc ggccggtacc ccgtctcaca actccca	37
		- •
<210>	9	
<211>		
<212>		
<213>	Artificial	
<223>	Primer VL1	
	9 :tct agaggagcct tttttttgga gat	33
gagecat	. coe agaggagoot coccegga gae	

<210> 10
<211> 26
<212> DNA
<213> Artificial

<223> Primer VL2

<400> 10
ctgagatgag tttttgttct gcggcc